Sponsor

Chemtura Corporation

Report for Inherent Biodegradation Test of MLA-3202

(Test Substance: MLA-3202)

Study No.: S2016NC020-03

Report No.: R2016NC020-03

Study Director: Zhou Linjun, Assistant Professor

Date of Report Completion

Nov. 18, 2016

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Content

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Statement of GLP Compliance

Study No.: S2016NC020-03

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According to "OECD Guidelines for testing of chemicals", "The guidelines for the testing of chemical (HJ/T 153-2004)" and "The guidelines of chemical testing good laboratory practices (HJ/T 155)" issued by State Environmental Protection Administration (SEPA) of the People's Republic of China, this experiment was conducted under CMA (China Metrology Accreditation) and CNAS (China National Accreditation Service for Conformity Assessment, CNAS) experimental conditions at our laboratory, and was performed in compliant with OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17). The experimental protocol was followed in the process of the experiment, and the present report has reflected the experimental results truly and correctly. This report was only responsible for the materials provided by sponsor.

Zhou Linjun — Nov. 18, 2016

Study Director: Date:

Shi Li Li — Nov. 18. 2016

Facility Manager: Date:

Quality Assurance Statement

Study No.: S2016NC020-03

Report No.: R2016NC020-03

The experiment was carried out strictly in accordance with the experimental protocol. It is hereby certified that what the present report describes has accurately reflected the raw data of the experiment.

During the on-site process inspections procedures applicable to this type of study were inspected.

The reporting date is the date of reporting to the Study Director. The QAU report was then forwarded to the Test Facility Manager. The dates of Quality Assurance inspection are given below.

Type of inspections	Phase/Process	Date		
		Start Inspection	End Inspection	Reporting
Study	Protocol	May 13, 2016	May 13, 2016	May 13, 2016
	Draft report Final report	Aug 17, 2016 Nov. 18, 2016	Aug 17, 2016 Nov. 18, 2016	Aug 17, 2016 Nov. 18, 2016
Process	Analysis of COD Chemical Analysis	Jul 13, 2016 Jul 20, 2016	Jul 13, 2016 Jul 20, 2016	Jul 13, 2016 Jul 20, 2016

Gino Min

Quality Assurance:

Nov 18, 2016

Date:

Study Details Page

Study number:	S2016NC020-03
Report number:	R2016NC020-03
Study title:	Inherent Biodegradation Test: Modified MITI Test (II)
Test substance①:	MLA-3202
Chemical name:	Amides, tallow, N,N-bis(2-hydroxypropyl)
CAS:	1454803-04-3
Chemical formula:	$C_{20}H_{39}NO_3$ to $C_{26}H_{51}NO_3$ (in H_2, C_2H_2 and C increments)
Molecular weight:	NA
Purity:	100 % (UVCB substance)
Lot number:	RC-1045
Re-certification date:	Apr. 11, 2017
Appearance:	Amber liquid
Storage conditions:	Airtight store in a cool ventilated dry place.
Water solubility	Trace (< 1 g/L)
Facility Manager:	Shi Lili, professor
Study Director:	Zhou Linjun, assistant professor Telephone: (+86) 25 8528 7057 Facsimile: (+86) 25 8547 6477 Email: zhoulj@nies.org
Location of study:	Key Lab of Pesticide Environmental Assessment and Pollution Control, MEP (PEAPC) Nanjing Institute of Environmental Sciences, MEP 8 Jiang-wang-miao Street, Nanjing 210042
Study dates:	
Study initial:	Jun. 01, 2016
Experiment starting:	Jun. 22, 2016
Experiment completion:	Jul. 20, 2016
Study completion:	Nov. 18, 2016

①Test substance information was provided by the sponsor, the unavailable information was marked as "NA" and certificate of analysis can be found in Appendix 1.

1 Summary

The inherent biodegradation test on the test substance (MLA-3202) was performed according to "The guidelines for the testing of chemicals" SEPA(HJ/T 153-2004), "The guidelines for the testing of chemicals, Degradation and Accumulation" (the 2nd edition) (Beijing: China Environment Press. 2013), and Procedure 302C of the 'Guidelines for Testing of Chemicals' of the OECD: "Inherent Biodegradability: Modified MITI Test (II)" (1981).

Test solutions were prepared in an inorganic salts medium, inoculated with a number of micro-organisms collected from 10 places in Nanjing city. During the test, the temperature was kept at (25±2) °C. The test was valid because the level of biodegradation of the reference substance sodium benzoate was 78.6% after 7 days (> 40%), and 84.9 % after 14 days (> 65%), and the recovery rate of residual amount of the test compound in the "abiotic control" is found to be more than 10% after 28 days.

The BOD results showed that inherent biodegradation of the test substance (MLA-3202) was 77.9% after 28 days based on the calculation of $ThOD_{NH3}$.

Based on the residue analysis, inherent biodegradation of the test substance (MLA-3202) was 100 % during the testing period.

Substances are considered to be "inherently biodegradable" if the inherent degradation rate is equal to or greater than 20% during the 28-day test period. MLA-3202 can therefore be considered inherently biodegradable.

2 Test Purpose

The purpose of this test was to evaluate the inherent biodegradability of organic chemicals via a 28-day test. In the test, the test substance (MLA-3202) and micro-organisms not adapted to the test substance were added into the aerobic, aqueous medium in BOD bottles respectively. Then the Biochemical Oxygen Demand (BOD) and residual chemicals in BOD bottles were measured during the 28-day period.

The study was designed to meet the requirements of following guidelines:

[1] HJ/T 153-2004, The guidelines for the testing of chemicals [S]. Beijing: SEPA, 2004

[2] CRC-MEP. The Guidelines for the Testing of Chemicals, Degradation and Accumulation [M]. 2nd edition. Beijing: China Environment Press. 2013: 74-81.

[3] GB/T 21801-2008, Chemical Inherent Biodegradation-Modified MITI Test (II) [S] Beijing: SAC, 2008.

[4] Guidelines for Testing of Chemicals, 302C, Inherent Biodegradability: Modified MITI Test (II). Paris: OECD, 1981.

Test solutions were prepared in an inorganic salts medium, inoculated with a number of micro-organisms collected from not less than 10 places in Nanjing city. The collected organisms were kept in BOD bottles in the dark at (25±2) °C.

Four groups ("abiotic control", "procedure control", "blank inoculum control", and "test") were set up simultaneously. The "abiotic control" contained deionised water and a measured amount of test substance in order to determine whether there were any abiotic losses of the test chemical during the testing period. The "procedure control" contained inoculated mineral salts medium and a measured amount of a reference substance to validate the test result. The "blank inoculum control" only contained inoculated mineral salts medium and the "test" contained inoculated mineral salts medium and a measured amount of the test substance.

The progress of degradation was followed by the determination of BOD in the "test" and the three "controls". Degradation was expressed as the ratio of the biochemical oxygen demand (BOD) and the chemical oxygen demand (COD) in order to evaluate the inherent biodegradability of chemical substance.

The inherent degradation rate was also expressed as percentage of initial concentration of test substance, for which a residue analysis of the test substance was performed at the end (28 d) of the test.

Substances are considered to be "inherently biodegradable" if the inherent degradation rate is equal to or greater than 20% during the 28-day test period.

3 Equipments &Materials

3.1 Equipments

BOD meter (OM3100, Ohkura, Japan)

BOD bottles (500 mL)

Analytical balance (MS105DU, METTLER TOLEDO, Switzerland)

Analytical balance (PL402-L, METTLER TOLEDO, Switzerland)

High-speed refrigerated centrifuge (CR22GII, HITACHI, Japan)

pH-electrodes (HQ40d, HACH, USA)

Water purification system (Milli-Q, Millipore, USA)

Pipette (Finnpipette, ThermoFisher, USA)

COD Analyzer (ET99718, Lovibond, Germany)

GC-FID (AGILENT 7820A, Agilent USA)

3.2 Reagents

Deionized water: Resistivity: 18.2 MΩ.cm

COD regent (0~150 mg/L)
Lot: P10A
Supplier: Lovibond

Reagents for the medium were all analytical pure.

Potassium di-hydrogen phosphate
Molecular formula: KH_2PO_4
Purity: ≥ 99.5%
Lot: 14101112154
Supplier: Nanjing Chemical Reagent Co., Ltd.

Dipotassium hydrogen phosphate
Molecular formula: K_2HPO_4
Purity: ≥ 98.0%
Lot: 20130322
Supplier: Sinopharm Chemical Reagent Co., Ltd.

Disodium phosphate dodecahydrate
Molecular formula: $Na_2HPO_4.12H_2O$
Purity: ≥ 99.0%
Lot: 13091111483
Supplier: Nanjing Chemical Reagent Co., Ltd

Ammonium chloride
Molecular formula: NH_4Cl
Purity: ≥ 99.5%
Lot: 13082011297
Supplier: Nanjing Chemical Reagent Co., Ltd.

Calcium chloride
Molecular formula: $CaCl_2$
Purity: ≥ 96.0%
Lot: 20130617
Supplier: Nanjing Chemical Reagent Co., Ltd.

Magnesium sulphate heptahydrate

Molecular formula: $MgSO_4.7H_2O$
Purity: ≥ 99.0%
Lot: 14052811195
Supplier: Nanjing Chemical Reagent Co., Ltd.

Iron (III) chloride hexahydrate
Molecular formula: $FeCl_3.6H_2O$
Purity: ≥ 99.0%
Lot: 20131017
Supplier: Sinopharm Chemical Reagent Co., Ltd.

Sodium benzoate
Molecular formula: $C_6H_5CO_2Na$
Purity: ≥ 99.5%
Lot: 20141121
Supplier: Sinopharm Chemical Reagent Co., Ltd.

Acetone
Molecular formula: C_3H_6O
Purity: ≥ 99.8%
Lot: K45960920
Supplier: MERCK KgaA

n-hexane
Molecular formula: C_6H_{12}
Purity: ≥ 98.0%
Lot: K47534591
Supplier: MERCK KgaA

Dichloromethane
Molecular formula: CH_2Cl_2
Purity: ≥ 99.5%
Lot: 1603001YT
Supplier: MERCK KgaA

4 Test Method

4.1 Preparation of the Inoculums

Activated sludge, surface soil and surface water were sampled from ten sites distributed in four districts throughout Nanjing city, such as Chengdong, Chengbei, Jiangxinzhou and Jiangning. 1 L of the sludge, soil and water were collected and mixed thoroughly together. After removing floating matter, the mixture was allowed to stand and then the supernatant was filtrated through filter paper. After that the filtrate was adjusted to pH 7.0 with sodium hydroxide or phosphoric acid. Finally an appropriate volume of the filtrate was transferred to a fill-and-draw activated sludge vessel and aerated for about 23.5 h (Batch No.: IN201605231).

Thirty minutes after stopping the aeration, about one third of the whole volume of

supernatant was discarded. Then an equal volume of synthetic sludge (a solution at pH 7.0 containing 0.1% each of glucose, peptone and potassium orthophosphate) was added into the settled material which was then aerated again. This procedure was repeated once per day during one month.

Before use the mixture was allowed to stand, and the supernatant was removed. A small quantity of sludge was taken to be centrifuged (2500 r/min×10 min) and then weighed. Then the sludge was dried in the oven and weighed again in order to calculate the content of dry sludge was 12.6%. At last 15.88 g of centrifuged sludge was diluted 0.5 L with basal culture medium (BSM) to get an activated sludge suspension with a concentration of 4000 mg/L (dry basis).

4.2 Preparation of the Test Medium

The Based Salt Medium (BSM) was prepared by adding 3 mL of each of the following stock solutions prepared in pre-aerated deionized water to 1 litre of deionized water.10 L BSM was prepared (Batch No.: BSM201606221).

Stock solution A	**g/L**
KH_2PO_4 (potassium dihydrogen phosphate)	8.50
K_2HPO_4 (dipotassium hydrogen phosphate)	21.75
$Na_2HPO_4.12H_2O$ (disodium phosphate dodecahydrate)	44.60
NH_4Cl (ammonium chloride)	1.70
The pH of this solution was 7.30	
Stock solution B	
$CaCl_2$ (calcium chloride)	27.50
Stock solution C	
$MgSO_4.7H_2O$ (magnesium sulphate heptahydrate)	22.50
Stock solution D	
$FeCl_3.6H_2O$ (iron (III) chloride hexahydrate)	0.25

4.3 Preparation of Solutions of the Test Substance

Test Substance was added to the test bottles directly.

A stock solution of the reference substance (sodium benzoate) at 1002 mg/L: 1.0022 g of reference substance was dissolved in 1 L volumetric flask with BSM.

4.4 Test Conditions

(1) Average concentration of test chemicals: 30 mg/L.

(2) Concentration of reference chemicals: 100 mg/L (W/V)

(3) Concentration of activated sludge in "test" and "blank inoculum control": 100 mg/L (W/V)

(4) Concentration of activated sludge in "reference": 30 mg/L (W/V)

(5) Test temperature: (25±2) °C

(6) Period: 28 days

(7) pH: 7.29-7.52

(8) Stir vigorously with mechanical stirrer.

4.5 Test Procedure

The following test substance will be added to the BOD bottles 1"abiotic control", bottles 2-4 "test":

Bottle	1	2	3	4
Test substance (g)	0.0092	0.0104	0.0108	0.0091
Concentration of test substance (mg/L)	30.7	34.7	36.0	30.3

Bottle 1 designated as "abiotic control" was then filled to a final volume of 300 mL with deionised water. Concentration of test chemical was 50 mg/L.

For bottles 2-4 designated as "test", 7.5 mL of activated sludge suspension were added to each of these after which they were filled to a final volume of 300 mL with BSM. The concentration of activated sludge was 100 mg/L and concentration of test chemical was 50 mg/L.

For bottle 5 designated as "procedure control", 30 mL stock solution of reference substance and 2.25 mL of activated sludge suspension were added. Bottle was then filled to a final volume of 300 mL with BSM. The concentration of activated sludge was 30 mg/L and concentration of the reference chemical was 100 mg/L.

For bottle 6 designated as "blank inoculum control", 7.5 mL of activated sludge suspension was added and bottle was then filled to a final volume of 300 mL with BSM. The concentration of activated sludge was 100 mg/L.

The equipment was then assembled, checked for air-tightness, stirrers were started and measurement of oxygen uptake under conditions of darkness began.

The temperature, the operation of the stirrer and recorder was checked daily. Any changes in colour of the contents of the vessels were recorded. The BOD for the six bottles were determined and recorded.

After the 28 days of testing, concentration of MLA-3202 in the testing bottles was analysed.

4.6 Analysis of COD

The COD was determined to substitute the ThOD which cannot be calculated as the test substance is mixture.

Stock solution of test substance (1150 mg/L): 0.0575 g test substance was weighted and made up to 50 mL with acetone.

30.0 mg/L and 50.0 mg/L samples were prepared by adding 52.2 μL and 87.0 μL of stock solution of the test substance (1150 mg/L) into test tubes, with solvent was dried, Then 2 mL COD reagents were added, and 3 parallel for each concentration. Blank control was prepared by above method expect for stock solution of the test substance substituted by solvent.

Heat all samples at 150°C for 120 minutes, and then determine the COD of samples after cool them to room temperature.

4.7 Chemical Analysis

(1) Preparation of test substance stock solutions

The standard stock solution I of the test substance (1152 mg/L) was prepared by dissolving 0.0576 g test substance (Purity: 100 %) into 50.0 mL with n-hexane.

The standard stock solution II of the test substance (100 mg/L) was prepared by drawing 8.68 mL the standard stock solution I (1152 mg/L) into 100 mL with n-hexane.

(2) Preparation of the test substance standard solutions

The standard stock solution I and II of the test substance was used to prepare the standard solutions. Details of the standard solutions are showed below:

Conc. of standard solution (mg/L)	Con. of the stock solution (mg/L)	The volume of the stock solution (mL)	Final volume after dilution (mL)
5.00	100	0.5	10
10.0	100	1.0	10
20.0	100	2.0	10
50.0	100	5.0	10
100	1152	0.868	10

(3) GC-FID conditions

Apparatus: Agilent 7820A (Agilent USA)

Column: HP-5 30 m × 320 μm×0.25 μm (Agilent USA)

Detector: Flame ionization detector (FID)

Injection volume: 1 μL

Injection temperature: 300 ℃

Oven temperature programme: 5 ℃/min from 200 to 280 ℃, 30 ℃/min to 300 ℃, keep 3 min

Detector temperature: 300 ℃

Helium flow rate 40 mL/min

Air flow rate 400 mL/min

Make-up flow rate (nitrogen): 16 mL/min

Retention time: test substance is about 3.2 min、3.5 min、3.6 min、4.5 min and 5.1 min (Figure 3)

(4) Sampling and analysis of test solution

Samples were taken from "abiotic control" bottle, "test" bottles and "blank inoculum control" bottle. For the "abiotic control" bottle, 300 mL samples were extracted with 300 mL dichloromethane for 15 min. Then, the samples were filtered using 0.22 μm millipore filters. First 5 mL was discarded. For the "test" bottles and "blank inoculum control" bottle, 100 mL samples were extracted with 100 mL dichloromethane for 20 min. Then, the dichloromethane was removed with the helping of the rotary evaporation apparatus. Finally,

the samples were diluted with n-hexane to 2 mL, and then filtered using 0.22 μm millipore filters.. The concentration of samples was determined with the analysis method mentioned above.

4.8 Test Validity

The test is considered to be valid, if biodegradation of the reference substance reached > 40% and > 65% on day 7 and day 14, respectively, and if the recovery rate of residual amount (experimental, mg) of the test compound in the "abiotic control" is found to be more than 10%.

5 Data Processing

5.1 Calculation of Theoretical Oxygen Demand

The theoretical oxygen demand (ThOD) of the test substance or reference substance with a molecular formula of

$$C_cH_hNa_{na}O_o$$

is calculated in the following way assuming that carbon is mineralised to CO_2, hydrogen to H_2O, chlorine to HCl and sodium to Na_2O, when N to NH_3:

$$ThOD_{NH_3} = \frac{16[2c + 1/2(h - hal - 3n) + 1/2na - o]}{MW} \text{ mg } O_2/\text{mg} \quad \ldots\ldots (1)$$

The $ThOD_{NH3}$ of reference substance is calculated to be 1.67 mg O_2/mg.

5.2 Calculation of BOD

The oxygen uptake (mg) by the test substance after a given time, corrected for that taken up by the blank inoculum control after the same time is divided by the weight of the test substance used. This yields the BOD expressed as mg oxygen/mg test substance as Formula (2) and procedure control as formula (3),

$$\text{BOD (mg } O_2\text{/mg test substance)} = \frac{T_{(n)} - C_{(n)}}{\text{mg (test substance) /L}} \quad \ldots\ldots(2)$$

$$\text{BOD (mg } O_2\text{/mg reference substance)} = \frac{T_{(n)} - 30/100 \times C_{(n)}}{\text{mg (reference substance) /L}} \quad \ldots\ldots(3)$$

Where:

$T_{(n)}$ = mg O_2/L content in the test or reference substance bottles on day n;

$C_{(n)}$ = mean mg O_2/L content in the inoculum control bottles on day n;

n = Day number sample measured.

30/100 = transforming factor, as concentration sludge in blank inoculum control was 100 mg/L, while concentration sludge in procedure control was 30 mg/L.

5.3 Calculation of Percentage Biodegradability

(1) Method for calculating the percentage biodegradation from BOD:

$$\%\,\text{deg radation} = \frac{BOD}{COD} \times 100 \quad \ldots\ldots(4)$$

BOD: Biological oxygen demand of the test compound measured on the BOD curve, mg/mg.

COD: Theoretical oxygen demand (theoretical, mg) required when the test compound is completely oxidised, mg/mg.

(2) Method for calculating the percentage degradation from the result of chemical analysis:

$$\text{Biodegradation}(\%) = \frac{S_b / m_b - S_a / m_a}{S_b / m_b} \times 100 \quad \text{.....(5)}$$

S_a: Concentration in the "test" after completion of the biodegradability test, mg/L.

S_b: Concentration in the "abiotic control" after completion of the biodegradability test, mg/L.

m_a: Added test substance in the "test" before test, mg.

m_b: Added test substance in the "abiotic control" before test, mg.

6 Test Results

6.1 Analytical Result of COD

COD results was shown in table 4, COD of the test substance was 2.64 mg O_2/mg based on the COD analysis.

6.2 Analytical Method of the Test Substance

(1) Specificity

Using analysis conditions mentioned above, the chromatographic peak of test substance were emerged at retention time tR=3.1 min, tR=3.4 min, tR=3.6 min, tR=4.5 min and 5.1min (Figure 3) whereas this chromatographic peak did not emerge in the "blank inoculum control" (Figure 7). Therefore, the GC-FID method was specific for test substance.

(2) Calibratrion curve

A series of standard solutions of test substance with concentration at 5.00, 10.0, 20.0, 50.0 and 100 mg/L were measured under the GC-FID using analysis conditions mentioned above. Concentrations of the test substance were quantified by GC-FID using five of the principal peaks which were considered representative of the different physic-chemical characteristics of the individual components making up the complex nature. An example chromatogram can be found in Figure 3. Based on the test result, linear regression equations was obtained with the peak areas vs. standard solution concentration, $A = 3978c + 515$, $r^2 = 0.9988$, where A represents peak area, and c is the concentration of the test item (mg/L) (See Figure 2). The results show that linearity for the concentration range of 5.00 mg/L to 100 mg/L is good.

(3) Precision

Under the above conditions, 50.0 mg/L solution of the test substance was analysed for 6 times, the results are shown in Table 5. The relative standard deviation was 1.80 %.

(4) Recovery test

The recovery samples with concentrations at 5.00 mg/L were prepared in triplicate by adding 86.8 μL of the standard stock solution I (1152 mg/L) to 0.50 mL of activated sludge suspension (4000 mg/L) and making it up to 20 mL with BSM. 20 mL of the recovery

samples were then extracted with 20 mL dichloromethane 15 min. Then, the dichloromethane was removed with the helping of the rotary evaporation apparatus. Finally, the samples were diluted with n-hexane to 2 mL, and then filtered using 0.22 μm millipore filters.The concentration of samples was determined with the analysis method mentioned above.

The recovery samples with concentrations at 30.0 mg/L were prepared in triplicate by adding 521 μL of the standard stock solution I (1152 mg/L) to 0.50 mL of activated sludge suspension (4000 mg/L) and making it up to 20 mL with BSM. 20 mL of the recovery samples were then extracted with 20 mL dichloromethane for 15 min. Then, the samples were filtered using 0.22 μm millipore filters. First 5 mL was discarded and the concentration of samples was determined with the analysis method mentioned above.

Measurements obtained from the recovery test were shown in Table 6. The results showed that mean recovery rate for 5.00 mg/L and 30.0 mg/L was 88.8% ~ 96.6% and 97.7% ~105%, the relative standard deviation was 4.22 % and 3.81%, respectively. Example chromatograms can be found in Figure 4.

(5) Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD was 2.00 mg/L (S/N≥3), and the LOQ was 5.00 mg/L (S/N≥10).

6.3 Inherent Biodegradation of Test Substance

Test solutions were prepared in an inorganic salts medium, inoculated with a number of micro-organisms collected from 10 places in Nanjing city. During the test, the temperature was kept at (25±2) °C. The test was valid because the level of biodegradation of the reference substance, sodium benzoate, was 78.6 % after 7 days (> 40%), and 84.9 % after 14 days (> 65%), and recovery rate of residual amount of the test compound in the "abiotic control" test was found to be more than 10% after 28 days.

The BOD results showed that inherent biodegradation of the test substance (MLA-3202) was 77.9 % after 28 days based on $ThOD_{NH3}$.

Based on the residue analysis, biodegradation of the test substance (MLA-3202) was 100 % during the testing period, please see Table 6 for details. Example chromatograms can be found in Figure 5 - 7.

Substances are considered to be "inherently biodegradable" if the inherent degradation rate is equal to or greater than 20% during the 28-day test period. MLA-3202 can therefore be considered inherently biodegradable.

7 GLP Compliance

We strictly comply with "The guidelines of chemical testing good laboratory practices (HJ/T 155)", Good Laboratory Practice (GB/T 22278/2008), China Metrology Accreditation (CMA) and China National Accreditation Service for Conformity Assessment (CNAS).

8 Deviations

None.

9 Health & Safety

In order for PEAPC to comply with Law of the People's Republic of China on the Prevention and Treatment of Occupational Diseases 2001, and the current Control of Substances Hazardous to Health Regulations, it is a condition of undertaking the study that the Sponsor provide PEAPC with all information available to it regarding known or potential hazards associated with the handling and use of any substance supplied by the Sponsor to PEAPC. The Sponsor also complied with all current legislation and regulations concerning shipment of substances by road, rail, sea or air.

Such information in the form of a completed PEAPC test item data sheet must be received at PEAPC before the test item can be handled in the laboratory.

10 Records & Documentation

All raw data arising from the performance of this study will remain the property of the Sponsor.

Records and documentation relating to this study (including the Study Protocol, raw data and a copy of the final report) will be maintained for a period of ten year from the date on which the Study Director signs the final report. Remaining samples will be retained by test facility in its archive for a period of one year from the date on which the Study Director signs the final report. After this one year, if no request of sponsor on return or further retention of the materials, the retained samples will be disposed as hazardous waste process.

Test report in 3 copies with original signatures will be provided. 1 of them will be retained by test facility in its archive, and the remaining 2 will be sent to sponsor. The Quality Assurance records relevant to this study will also be archived.

11 References

[1] HJ/T 153-2004, The guidelines for the testing of chemicals [S]. Beijing: SEPA, 2004

[2] CRC-MEP. The Guidelines for the Testing of Chemicals, Degradation and Accumulation [M]. 2nd edition. Beijing: China Environment Press. 2013: 74-81.

[3] GB/T 21801-2008, Chemical Inherent Biodegradation-Modified MITI Test (II) [S] Beijing: SAC, 2008.

[4] Guidelines for Testing of Chemicals, 302C, Inherent Biodegradability: Modified MITI Test (II). Paris: OECD, 1981.

Tables

Table 1 Results of O_2 Uptake during 28 Days Period

Time (d)	O_2 uptake (mg/L)					
	"abiotic control"	"test"			"procedure control"	"blank inoculum control"
	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Bottle 5	Bottle 6
0	0	0	0	0	0	0
1	0	8.64	8.52	6.83	0	0
2	0	13.6	13.7	12.7	85.6	2.19
3	0	32.4	33.8	26.5	112	4.38
4	0	38.3	39.5	34.1	118	5.11
5	0	43.0	44.4	41.5	124	5.65
6	0	46.5	48.1	43.4	130	6.09
7	0	49.2	51.9	46.3	134	7.24
8	0	54.1	57.0	47.7	138	8.34
9	0	57.2	60.7	51.8	141	8.79
10	0	62.0	68.5	58.6	141	9.26
11	0	66.6	72.8	63.4	142	10.1
12	0	69.9	76.2	66.6	144	10.7
13	0	71.4	78.6	67.8	145	11.4
14	0	73.3	80.8	68.8	145	12.3
15	0	74.8	82.4	70.7	147	12.4
16	0	78.9	86.8	74.0	150	15.0
17	0	82.8	91.2	77.5	153	18.0
18	0	84.3	93.2	80.7	155	20.1
19	0	84.3	93.2	81.2	155	20.1
20	0	84.3	93.2	81.2	155	20.1
21	0	84.3	93.2	81.2	155	20.1
22	0	84.3	93.5	81.4	155	20.1
23	0	84.3	94.9	81.4	155	20.1
24	0	85.1	96.9	81.4	155	20.1
25	0	85.9	97.9	81.4	155	20.1
26	0	86.3	99.0	81.4	155	20.1
27	0	87.9	100.6	81.9	155	21.0
28	0	88.2	101.7	81.9	155	21.2

Table 2 Cumulative Biodegradation (%)

Time (d)	Cumulative Biodegradation (%)				
	"test"				"procedure control"
	Bottle 2	Bottle 3	Bottle 4	Mean	Bottle 5
0	0	0	0	0	0
1	9.44	8.96	8.53	8.97	0
2	12.4	12.1	13.1	12.5	50.8
3	30.6	30.9	27.6	29.7	66.1
4	36.3	36.2	36.2	36.2	69.5
5	40.8	40.8	44.7	42.1	73.0
6	44.1	44.2	46.5	44.9	76.7
7	45.9	47.0	48.8	47.2	78.6
8	50.0	51.2	49.1	50.1	81.2
9	52.9	54.6	53.7	53.7	82.9
10	57.7	62.3	61.6	60.5	82.9
11	61.8	66.0	66.6	64.8	83.5
12	64.7	68.9	69.9	67.8	84.3
13	65.6	70.7	70.4	68.9	85.0
14	66.6	72.0	70.6	69.7	84.9
15	68.2	73.7	72.9	71.6	85.7
16	69.9	75.5	73.7	73.0	87.1
17	70.8	77.1	74.3	74.1	88.4
18	70.1	76.9	75.7	74.3	89.0
19	70.1	76.9	76.4	74.5	89.0
20	70.1	76.9	76.4	74.5	89.0
21	70.1	76.9	76.4	74.5	89.0
22	70.1	77.2	76.6	74.7	89.0
23	70.1	78.8	76.6	75.2	89.0
24	71.1	80.8	76.6	76.2	89.0
25	71.9	81.9	76.6	76.8	89.0
26	72.4	83.0	76.6	77.3	89.0
27	73.1	83.8	76.1	77.7	88.9
28	73.2	84.7	75.8	77.9	88.8

Table 3 pH of the Test Solutions

Sample		pH	
		0 d	28 d
"abiotic control"	Bottle 1	7.35	7.48
"test"	Bottle 2	7.31	7.46
	Bottle 3	7.32	7.39
	Bottle 4	7.35	7.41
"procedure control"	Bottle 5	7.32	7.52
"blank inoculum control"	Bottle 6	7.29	7.35

Table 4 COD of the Test Substance

Concentration (mg/L)	Total COD (mg/L)	Test substance COD (mg O_2/mg)
30.0	79	2.63
30.0	81	2.70
30.0	84	2.80
50.0	131	2.62
50.0	127	2.54
50.0	128	2.56
Average		2.64

Table 5 Precision of the GC-FID method for the test substance

Nominal Concentration (mg/L)	Peak Areas（Hz.s）	RSD （%）
50.0	204475	2.44
	202986	
	202034	
	210083	
	207479	
	195671	

Table 6 Recovery of the Test Substance in Recovery Samples (GC-FID)

Nominal Concentration (mg/L)	Measured Concentration (mg/L)	Recovery Rate (%)	Mean Recovery Rate (%)	RSD (%)
5.00	4.83	96.6	92.5	4.22
	4.61	92.3		
	4.44	88.8		
30.0	31.5	105	102	3.81
	31.1	104		
	29.3	97.7		

Table 7 Residual Results and Degradation of the Test Substance

Sample	Time(d)	Bottle	Concentration (mg/L)	Degradation (%)
"abiotic control"	28	1	30.6	—
"blank inoculum control"	28	6	ND*	—
"test"	28	2	ND*	100
		3	ND*	100
		4	ND*	100
		Average	ND*	100

: Not detected, since below the detection limit of 2 mg/L of instrument.

Figures

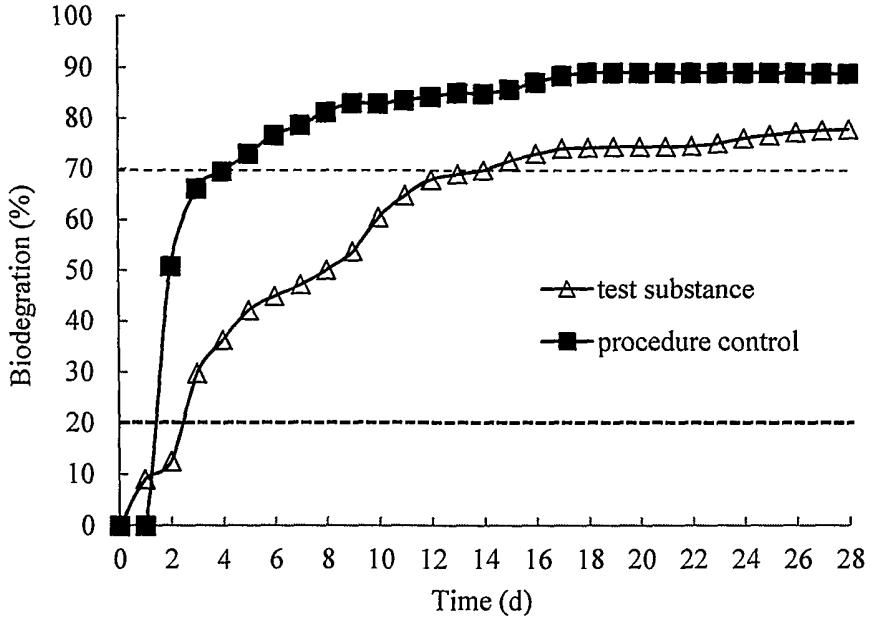

Figure 1 Biodegradation Curves

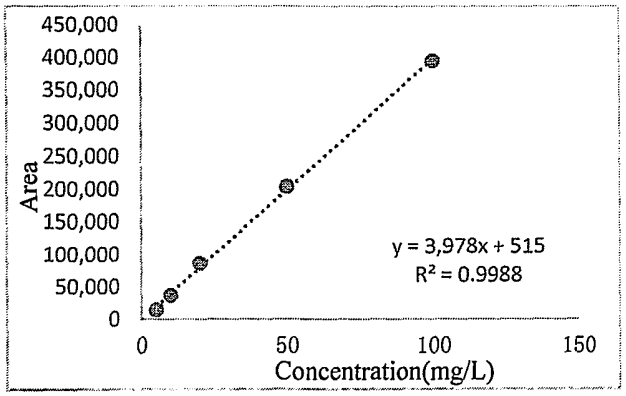

Figure 2 Calibration Curve of Test Substance

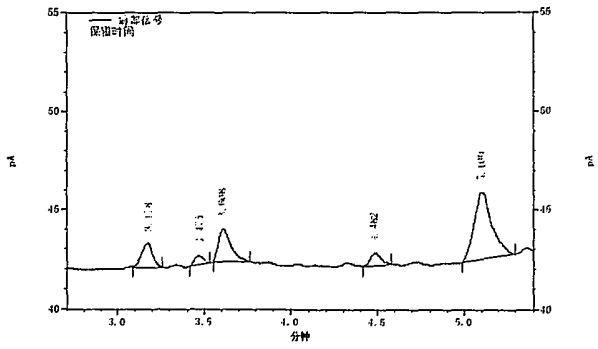

Figure 3 GC-FID Chromatogram of Test Substance Standard Solution (50.0 mg/L)

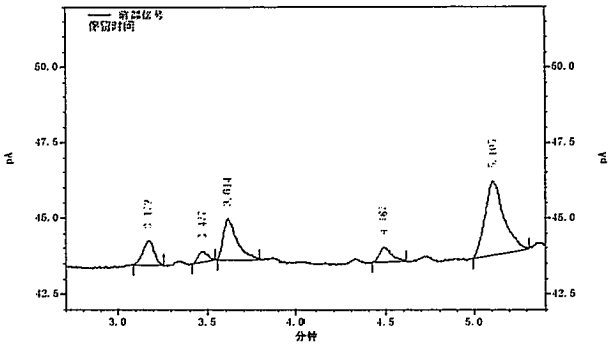

Figure 4 GC-FID Chromatogram of Recovery Sample 30.0 mg/L

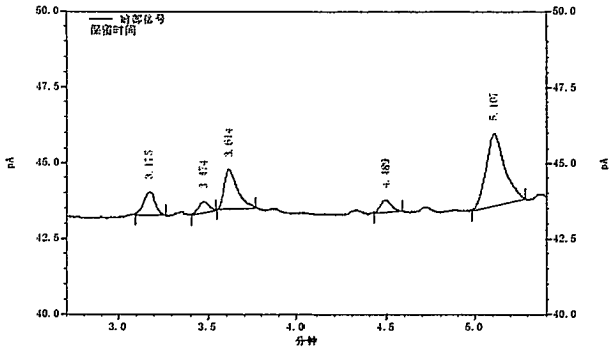

Figure 5 GC-FID Chromatogram of Abiotic Control Sample at 28 d

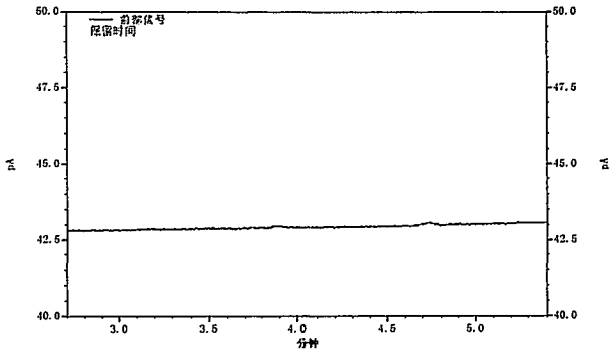

Figure 6 GC-FID Chromatogram of the Test Sample at 28 d (Concentrated 50 time)

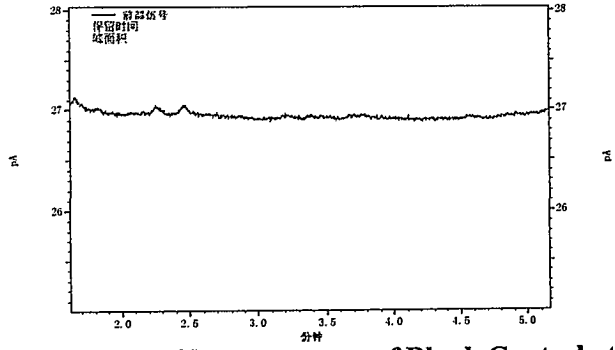

Figure 7 GC-FID Chromatogram of Blank Control at 28 d

Appendix

Appendix 1 Certificate of Analysis of the Test Substance

Chemtura Corporation
12 Spencer St
Naugatuck, CT 06770

Analytical Services
www.chemtura.com

Certificate of Purity

Customer: Support for Toxicology Studies

Test Substance Name: MLA3202; Amides, tallow, N,N-bis(2-hydroxypropyl)

Physical Appearance: Liquid

CAS No.: 1454803-04-3

Ref. or Lot Number: RC-1045

Date of Analysis: revised March 18, 2016 (original issue March 7, 2016)

Percent Composition	Monoisotopic Mass (daltons)	Formula	Structure/ Identity
33.1	397.4	$C_{24}H_{47}NO_3$	C18:1 (oleic) tallow amides, N,N-bis(2-hydroxypropyl)
22.9	371.3	$C_{22}H_{45}NO_3$	C16:0 (palmitic) tallow amides, N,N-bis(2-hydroxypropyl)
13.6	395.4	$C_{24}H_{45}NO_3$	C18:2 (linoleic) tallow amides, N,N-bis(2-hydroxypropyl)
11.0	399.4	$C_{24}H_{49}NO_3$	C18:0 (stearic) tallow amides, N,N-bis(2-hydroxypropyl)
6.0	369.3	$C_{22}H_{43}NO_3$	C16:1 (palmitoleic) tallow amides, N,N-bis(2-hydroxypropyl)
3.2	419.3	$C_{26}H_{45}NO_3$	C20:4 (eicosatetraenoic) tallow amides, N,N-bis (2-hydroxypropyl)
2.0	393.3	$C_{24}H_{43}NO_3$	C18:3 (linolenic) tallow amides, N,N-bis(2-hydroxypropyl)
1.5	282.3	$C_{18}H_{34}O_2$	C18:1 (oleic) acid
1.1	421.4	$C_{26}H_{47}NO_3$	C20:3 (eicosatrienoic) tallow amides, N,N-bis (2-hydroxypropyl)
5.6			Sum of residual components (< 1% each)
100.0			Total

Blake Lewis 3/7/16
Blake Lewis Date
Analytical REACh Scientist, Analytical Services

Colin Moore for AJN 3/7/16
Albert J. Nitowski Date
Sr. Technology Manager
Analytical and Lab Support Services